Original Article

DOI: 10.7860/JCDR/2026/77490.22446

Antioxidant Therapy using Astaxanthin and Quercetin against Chloramphenicol-induced Hepatotoxicity in Wistar Rats: A Randomised Controlled In-vivo Interventional Study

Complementary/Alternative Medicine Section

BRIJESH KUMAR VERMA[1], JAIMIN CHAUDHARI[2], DEBASHIS BANERJEE[3]

ABSTRACT

Introduction: Drug-induced Liver Injury (DILI) remains a major clinical concern, particularly with antibiotics like chloramphenicol, which are known to cause oxidative stress and hepatotoxicity. Mitochondrial dysfunction is increasingly recognised as a key mechanism in such liver damage.

Aim: To evaluate the protective effects of the antioxidants astaxanthin and quercetin against chloramphenicol-induced hepatotoxicity in Wistar rats by assessing biochemical markers of oxidative stress.

Materials and Methods: The present randomised controlled, in-vivo interventional study was conducted at Zydus Research Centre, Gujarat, India, from May to July 2023. Twenty-four healthy male Wistar rats (180-200 g) were randomly assigned into four groups (n=6 each): control, chloramphenicol-only, chloramphenicol + astaxanthin, and chloramphenicol + quercetin. Chloramphenicol was administered intraperitoneally (25 mg/kg/day for 14 days), followed by oral antioxidant therapy for 14 days (Astaxanthin: 20 mg/kg; Quercetin: 30 mg/kg). Blood samples were collected on days 0, 15, and 30 to assess Glutathione (GSH) and Nitric Oxide (NO) levels. Statistical analysis was performed using Analysis of Variance (ANOVA) and Sidak's multiple comparisons test (GraphPad Prism v8.0.2), with $p<0.05$ considered significant.

Results: Chloramphenicol significantly reduced GSH and elevated NO levels ($p<0.01$), indicating oxidative stress. Both antioxidants mitigated these effects, with Quercetin showing slightly superior efficacy in restoring GSH levels.

Conclusion: Astaxanthin and Quercetin exhibit significant hepatoprotective effects against chloramphenicol-induced oxidative damage. Further research is warranted to explore dose optimisation, long-term effects, and translation to clinical settings.

Keywords: Glutathione, Liver diseases, Mitochondrial dysfunction, Nitric oxide, Oxidative stress

INTRODUCTION

The liver, central to medication metabolism, is particularly susceptible to pharmaceutical-induced damage. Various pharmacological agents, including antibiotics, can induce liver damage, with antibiotics consistently identified as the primary culprits causing idiosyncratic liver injury. Antibiotics also emerge as a significant contributor to Acute Liver Failure (ALF) [1-3]. DILI is reported by almost all classes of drugs including antibiotics [4].

This investigation focuses on chloramphenicol which is, acknowledged for efficacy but is notorious for side-effects, particularly hepatotoxicity. Chloramphenicol is a broad-spectrum antibiotic that works by binding to the 50S subunit of the 70S ribosome, thereby inhibiting microbial protein synthesis. Despite its serious side-effects, which include haematological, gastrointestinal, and neurological issues, as well as gray syndrome in newborns, it remains widely used in many parts of the world for treating life-threatening infections like typhoid fever and meningitis. The highest concentrations chloramphenicol is found in the kidney, liver, and bile and approximately half of plasma chloramphenicol bound to albumin [5,6].

The study was performed to investigate the toxic effects of chloramphenicol on the liver and kidney in Wistar rats with 25 mg/kg of chloramphenicol dose which causes the liver toxicity [7]. Recently chloramphenicol has also been considered in the treatment of vancomycin-resistant *Enterococcus faecium* bacteraemia, an infection with limited therapeutic options. The drug has gained wide acceptance in the Third World countries particularly because it is cheap and effective [8,9].

The study aimed to mitigate chloramphenicol-induced liver toxicity using a combination therapy of two potent antioxidants, Astaxanthin and Quercetin. Astaxanthin, a powerful carotenoid derived mainly from marine organisms, is significantly more potent than β-carotene and vitamin E. It has shown preventive and therapeutic effects on various liver conditions, including fibrosis, tumors, Ischaemia-reperfusion injury, and non-alcoholic fatty liver disease. Astaxanthin modulates multiple signaling pathways, inhibits apoptosis and autophagy, and provides substantial protection against liver Ischaemia-reperfusion injury [10].

Astaxanthin is an oxygen-containing carotenoid mainly sourced from marine organisms, known for its strong antioxidant properties. It is widely utilised in medicine, healthcare products, and cosmetics. Research has highlighted its significant preventive and therapeutic effects on various liver diseases, including liver fibrosis, non-alcoholic fatty liver disease, liver cancer, and liver injuries caused by drugs and Ischaemia. These beneficial effects are attributed to its antioxidant and anti-inflammatory actions, as well as its ability to regulate multiple signaling pathways [11-14]. Quercetin, a widely prevalent flavonoid found in various plant parts, is renowned for its antioxidant, anticarcinogenic, anti-inflammatory, immunomodulatory, cardioprotective, and bacteriostatic properties. Studies on rats have shown that nano-liposomal Quercetin effectively protects against acute liver injury, highlighting its potential as a novel hepatoprotective and therapeutic agent for individuals with liver diseases [15-17].

NO and GSH are easy and reliable ways to identify the toxicity in rats. Studies indicate that GSH-containing dinitrosyl iron complexes have antioxidant properties, reducing oxidative stress and offering protection against toxicity. NO helps modulate

oxidative metabolism, thereby reducing the harmful effects of Reactive Oxygen Species (ROS). NO is generated during the chemical reduction of nitrofuran antibacterial drugs, detected polarographically, and linked to their antibacterial activity. This is because NO forms peroxynitrite anion, a cytotoxic compound that disrupts the electron-transfer chain in microorganisms, impacting their metabolism. Additionally, NO, a Reactive Nitrogen Species (RNS) is vital in physiological processes like vasodilation, immune response, and neurotransmission. By interacting with ROS and RNS, NO helps mitigate oxidative stress [18-22].

GSH is a powerful antioxidant found in cells, and it plays a key role in detoxification, antioxidant defense, and regulation of cellular redox status. GSH directly neutralises ROS and RNS, preventing cellular damage. It also works in conjunction with enzymes like GSH peroxidase to break down peroxides. Research has demonstrated that exogenous dinitrosyl iron complexes with GSH ligands can significantly reduce oxidative stress markers in rat blood, with an optimal dose range of 0.30-0.45 mM. This combined action helps protect cells from oxidative damage and supports overall cellular health [23,24].

The combination of NO and GSH can provide a synergistic effect in reducing oxidative stress and toxicity. GSH-containing dinitrosyl iron complexes have been shown to exhibit an antioxidant effect, reducing lipid peroxidation and maintaining the balance of oxidative stress markers [25,26].

An in-vitro investigation using human liver cells (HepG2) was conducted to assess mitochondrial toxicity through respiratory studies, ROS analysis, and gene expression studies. The results indicate that Astaxanthin and Quercetin have therapeutic efficacy in alleviating chloramphenicol-induced liver toxicity [10]. To validate these findings, this study was extended to in-vivo models. The in-vivo study was guided by clearly defined research objectives, described below, to systematically address the study's hypothesis and anticipated outcomes.

The present study aimed to investigate the hepatotoxic effects of chloramphenicol and evaluate the protective role of antioxidants Astaxanthin and Quercetin in Wistar rats.

The primary objective was to assess the changes in GSH and NO levels as biomarkers of oxidative stress following antioxidant treatment. The secondary objective was to compare the hepatoprotective efficacy of Astaxanthin and Quercetin and to evaluate their potential as therapeutic agents for chloramphenicol-induced liver injury.

Hypotheses:

- **Null Hypothesis (H_0):** Antioxidants Astaxanthin and Quercetin have no significant effect on chloramphenicol-induced oxidative liver damage in Wistar rats.
- **Alternate Hypothesis (H_1):** Antioxidants Astaxanthin and Quercetin significantly reduce chloramphenicol-induced oxidative liver damage in Wistar rats.

MATERIALS AND METHODS

The present randomised controlled in-vivo interventional study was conducted after obtaining required ethical approval from IAEC (ZRC/DMPK/BP/056/04-2K23) at the Zydus Research Centre, Gujarat, India, from May to July 2023. All the experiments were conducted in accordance with the guidelines and regulations set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and approved by the Institutional Animal Ethics Committee (IAEC).

Chemical and reagents: For the quantitative determination of NO (Catalog #: KGE001) and GSH (Catalog Number: 7511-100-K) R&D Systems, Inc. (USA) kit has been used. Chloramphenicol, and antioxidants (Astaxanthin and Quercetin) were purchased from Sigma-aldrich.

Animal model: Twenty-four Wistar rats, aged 6-8 weeks, weighing between 180-200 grams [27-29].

Inclusion criteria: Healthy male Wistar rats (180-200 g), aged 6-8 weeks.

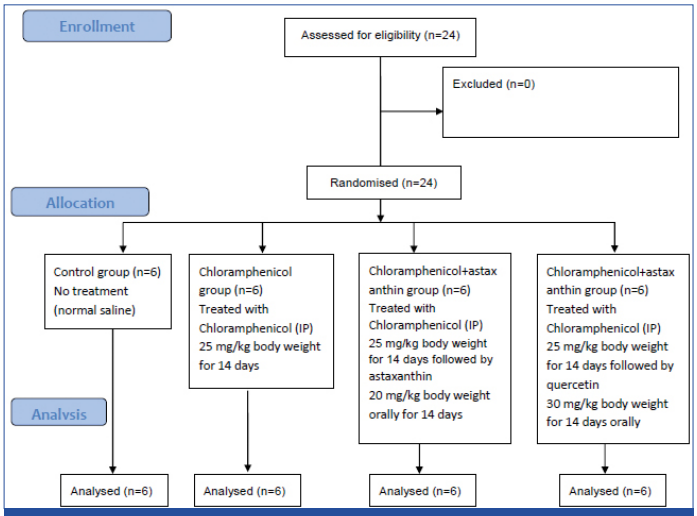
Exclusion criteria: Rats with signs of illness or outside the defined weight range (±10%) [29].

Acclimatisation: Allowed for one week to acclimate under standard laboratory conditions (12-hour light/dark cycle, 22±2°C, 60-70% humidity) with free access to food and water [29].

Experimental groups: The animals were randomly assigned into four experimental groups (I-IV) of six animals each [Table/Fig-1]. The doses were decided based on the available literature and were delivered in 1 mL solution of distilled water once. The animals of each group were treated as presented below:

- Control group (n=6): No treatment (normal saline).
- Chloramphenicol group (n=6): Treated with Chloramphenicol (25 mg/kg body weight, intraperitoneally) for 14 days [7].
- Chloramphenicol+astaxanthin group (n=6): Treated with Chloramphenicol (25 mg/kg body weight, intraperitoneally) for 14 days, followed by Astaxanthin (20 mg/kg body weight, orally) for 14 days [30].
- Chloramphenicol+quercetin group (n=6): Treated with chloramphenicol (25 mg/kg body weight, intraperitoneally) for 14 days, followed by quercetin (30 mg/kg body weight, orally) for 14 days [31].

Sample collection: Blood samples were collected from the retro-orbital plexus on day 0 (before treatment), day 15 (after Chloramphenicol treatment), and day 30 (after antioxidant treatment).



[Table/Fig-1]: CONSORT flowchart.

Study Procedure

Biochemical analysis: GSH is a key antioxidant that neutralises free radicals, it plays a vital role in maintaining cellular health and function. Changes in GSH levels can indicate cellular damage or the effectiveness of antioxidant treatments in protecting cells. An increase in GSH levels after antioxidant treatment suggests that the antioxidant is effective in reducing oxidative stress.

NO also plays a significant role in various physiological processes, including vasodilation, immune response, and neurotransmission. NO levels can indicate changes in oxidative stress and inflammation. Elevated NO levels can be a sign of increased oxidative stress or an inflammatory response, which might not be fully captured by GSH levels alone [25,26].

GSH assay: The method for measuring GSH levels in blood samples involves using a commercially available GSH assay kit (R&D Systems) based on the 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) reduction method. The assay employs an enzymatic recycling method where

glutathione reductase converts oxidised glutathione (GSSG) to its reduced form (GSH), which then reacts with DTNB to produce a yellow compound that absorbs light at 405 nm. The production rate of this compound is directly proportional to the GSH concentration. For blood samples, blood is collected in heparin tubes, treated with metaphosphoric acid, and centrifuged. Samples are then assayed in triplicate, diluted with assay buffer, and mixed with the glutathione reductase reaction mix. Absorbance is measured at 405 nm at 2-minute intervals over 10 minutes to quantify GSH levels [25].

NO assay: The NO assay measures NO levels by determining nitrite/nitrate concentrations in plasma using a commercially available NO assay kit. The assay involves converting nitrate to nitrite, then measuring total nitrite by subtracting the endogenous nitrite concentration. The procedure includes preparing all reagents, standards, and samples, adding reaction diluent to blank wells, and adding Nicotinamide Adenine Dinucleotide Hydride (NADH) and diluted nitrate reductase to each well. The mixture is incubated and then treated with Griess reagent I and II. After a final incubation at room temperature, the optical density is measured at 540 nm using a microplate reader. This method provides a reliable means of quantifying NO levels in biological samples [26].

STATISTICAL ANALYSIS

All statistical analyses were performed using GraphPad Prism software, version 8.0.2 (GraphPad Software, San Diego, CA, USA). Statistical comparisons between groups were conducted using one-way ANOVA, followed by Sidak's multiple comparisons test for multiple comparisons. The significance level of $p<0.05$ was considered statistically significant. Data are presented as mean±Standard Deviation (SD) unless otherwise specified.

RESULTS

Both antioxidants, Astaxanthin and Quercetin, significantly mitigated the oxidative stress and toxicity induced by chloramphenicol, which is evident by observed recovery of GSH levels and reduction in NO levels. This demonstrated that both the antioxidants have hepatoprotective effects against Chloramphenicol-induced toxicity [Table/Fig-2,3].

GSH Levels (nmol/mg)									
Group I: Control Group (n=6)					Group II: Chloramphenicol Group (n=6)				
R1	R2	R3	Mean GSH Levels	SD	R1	R2	R3	Mean GSH Levels	SD
8.5	8.3	8.8	8.53	0.3	4.1	3.9	4.3	4.10	0.2
7.6	8.1	8.4	8.05	0.4	3.4	3.8	4.2	3.80	0.4
8.8	9.3	9.6	9.23	0.4	4.0	4.3	4.6	4.30	0.3
7.2	7.8	8.4	7.82	0.6	3.6	4.2	4.4	4.07	0.4
8.6	8.7	8.9	8.74	0.2	4.1	4.2	4.3	4.20	0.1
8.4	9.2	9.4	9.01	0.5	3.7	4.0	4.3	4.01	0.3
GSH Levels (nmol/mg)									
Group III: Chloramphenicol+Astaxanthin (n=6)					Group IV: Chloramphenicol+Quercetin (n=6)				
R1	R2	R3	Mean GSH Levels	SD	R1	R2	R3	Mean GSH Levels	SD
6.5	8.0	7.0	7.17	0.8	7.6	7.8	8.0	7.81	0.2
7.2	7.5	7.8	7.50	0.3	7.3	7.6	7.9	7.60	0.3
6.6	6.9	7.2	6.90	0.3	7.5	7.9	8.3	7.90	0.4
7.5	7.3	7.1	7.31	0.2	7.3	7.7	8.1	7.70	0.4
6.9	7.1	7.3	7.10	0.2	7.8	8.0	8.2	8.01	0.2
7.0	7.4	7.8	7.40	0.4	7.6	7.8	8.0	7.81	0.2

[Table/Fig-2: Observed GSH levels in different treatment groups.

A. Baseline levels (before treatment):

Control group (n=6, total replicates=18): Initially, the mean value of three replicates from a single animal was presented to illustrate variation in relation to the observed values from the remaining five animals within the same group.

- GSH levels: 8.56 nmol/mg (normal range: 5-10 nmol/mg protein).
- NO levels: 15.47 µM (normal range: 10-20 µM).

B. After chloramphenicol treatment (day 15):

Chloramphenicol group (n=6, total replicates=18):

- GSH Levels: 4.08 nmol/mg (significant decrease, $p<0.01$ vs. control).
- NO Levels: 25.59 µM (significant increase, $p<0.01$ vs. control).

C. After antioxidant treatment (day 30):

- Chloramphenicol+Astaxanthin group (n=6, total replicates=18):
- GSH Levels: 7.23 nmol/mg (significant recovery, $p<0.05$ vs. Chloramphenicol group).
- NO Levels: 17.06 µM (significant reduction, $p<0.05$ vs. Chloramphenicol group).
- Chloramphenicol+Quercetin Group (n=6 total replicates=18):
- GSH Levels: 7.80 nmol/mg (significant recovery, $p<0.05$ vs. Chloramphenicol group).
- NO Levels: 16.50 µM (significant reduction, $p<0.05$ vs. Chloramphenicol group).

NO Levels (µM)									
Group I: Control Group (n=6)					Group II: Chloramphenicol Group (n=6)				
R1	R2	R3	Mean NO Levels	SD	R1	R2	R3	Mean NO Levels	SD
15.5	15.8	15.6	15.63	0.2	25.2	25.4	25.6	25.40	0.2
14.0	14.8	15.6	14.80	0.8	25.3	26.0	27.3	26.21	1.0
15.0	15.2	15.4	15.20	0.2	24.1	24.8	25.6	24.83	0.8
15.5	16.3	17.1	16.30	0.8	25.5	25.7	25.9	25.70	0.2
14.5	14.7	14.9	14.70	0.2	24.9	25.1	25.3	25.10	0.2
15.8	16.2	16.6	16.20	0.4	26.1	26.3	26.5	26.30	0.2
NO Levels (µM)									
Group III: Chloramphenicol+Astaxanthin (n=6)					Group IV: Chloramphenicol+Quercetin (n=6)				
R1	R2	R3	Mean NO Levels	SD	R1	R2	R3	Mean NO Levels	SD
16.9	17.1	17.3	17.10	0.2	16.2	16.4	16.6	16.40	0.2
16.2	16.8	17.4	16.80	0.6	15.8	16.2	16.6	16.20	0.4
17.2	17.4	18.0	17.54	0.4	16.5	16.7	16.9	16.70	0.2
16.7	16.9	17.1	16.90	0.2	16.2	16.3	17.0	16.51	0.4
17.2	17.2	17.4	17.28	0.1	16.1	16.3	16.5	16.30	0.2
16.1	17.3	16.9	16.77	0.6	16.6	16.8	17.2	16.87	0.3

[Table/Fig-3]: Observed Nitric Oxide (NO) levels in different treatment groups. R: Technical Replicates, n=Biological Replicates SD=Standard deviation, GSH: Glutathione; NO: Nitric Oxide; Values are expressed as mean±SD (n=6). GSH: Glutathione; NO: Nitric Oxide. Statistical analysis was performed using one-way ANOVA; *p<0.05 vs. control, †p<0.05 vs. chloramphenicol group.

Data expressed as mean±SD representing the statistical outcomes of Glutathione (GSH) levels is shown in [Table/Fig-4].

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant	Summary	p-value
Control Group vs. Chloramphenicol Group	4.467	3.737 to 5.197	Yes	****	<0.0001
Control Group vs. Chloramphenicol + AXN	1.300	0.1513 to 2.449	Yes	*	0.0305

Control Group vs. Chloramphenicol + QRN	0.7333	-0.01834 to 1.485	No	Ns	0.0550
Chloramphenicol Group vs. Chloramphenicol + QRN	-3.733	-3.867 to -3.600	Yes	****	<0.0001
Chloramphenicol Group vs. Chloramphenicol + AXN	-3.167	-3.802 to -2.531	Yes	****	<0.0001

[Table/Fig-4]: Presentation of statistical outcome of Glutathione (GSH) levels.
Note: Data analysed using One-way repeated measures ANOVA. F (1.343, 6.714) =237.6, p<0.0001. Sidak's multiple comparisons test was applied for multiple comparisons; Summary indicators: ns=not significant (p>0.05), *=p<0.05, **=p<0.01, ***=p<0.001, ****=p<0.0001; GSH: Glutathione; AXN: Astaxanthin; QRN: Quercetin. p<0.05 considered statistically significant

Data expressed as mean±SD representing the statistical outcomes of Nitric Oxide (NO) levels is shown in [Table/Fig-5] .

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant	Summary	p-value
Control Group vs. Chloramphenicol Group	-10.08	-11.15 to -9.015	Yes	****	<0.0001
Control Group vs. Chloramphenicol+ AXN	-1.567	-2.970 to -0.1630	Yes	*	0.0323
Control Group vs. Chloramphenicol+ QRN	-1.017	-1.945 to -0.08784	Yes	*	0.0349
Chloramphenicol Group vs. Chloramphenicol+ QRN	9.067	8.097 to 10.04	Yes	****	<0.0001
Chloramphenicol Group vs. Chloramphenicol+ AXN	8.517	7.122 to 9.912	Yes	****	<0.0001

[Table/Fig-5]: Presentation of statistical outcome of Nitric Oxide (NO) levels.
Note: Data analysed using One-way repeated measures ANOVA. F (1.854, 9.269)=580.5, p<0.0001. Sidak's multiple comparisons test was applied for multiple comparisons; Summary indicators: ns=not significant (p>0.05), *=p<0.05, **=p<0.01, ***=p<0.001, ****=p<0.0001; NO: Nitric Oxide; AXN: Astaxanthin; QRN: Quercetin. p<0.05 considered statistically significant

DISCUSSION

The present study elucidates the hepatoprotective efficacy of Astaxanthin and Quercetin against chloramphenicol-induced oxidative liver damage, a condition not extensively explored despite chloramphenicol's continued use in resource-limited settings [6-8]. The present study findings are consistent with previous reports suggesting that both compounds exert antioxidative effects by modulating redox balance and suppressing reactive species [10-13,15-17].

Astaxanthin also showed significant protection against chloramphenicol-induced hepatotoxicity. Its mechanism is believed to involve modulation of mitochondrial pathways and inhibition of apoptosis, which has been substantiated in models of liver f ibrosis, NAFLD, and Ischaemia-reperfusion injury [12-14,32,33]. For instance, Yamashita E reported that Astaxanthin improves mitochondrial integrity, thus reducing hepatic ROS generation [11], a key contributor to chloramphenicol toxicity [7,9]. Quercetin, a flavonoid with known antioxidant and anti-inflammatory properties, significantly improved oxidative stress parameters in our model, as reflected by restored GSH and reduced NO levels. This aligns with earlier studies where Quercetin attenuated oxidative injury in models of Non-Alcoholic Fatty Liver Disease (NAFLD) and cholestatic liver damage through modulation of autophagy and inflammatory signaling pathways [34,35]. Similarly, Liu X et al., demonstrated Quercetin's hepatoprotective role against acute liver injury by encapsulating it in nano liposomes, improving its bioavailability and efficacy [15].

Interestingly, in our study, while both antioxidants significantly reversed oxidative markers, Quercetin exhibited slightly superior efficacy in restoring GSH levels. This may be attributed to Quercetin's dual activity as both a direct scavenger of ROS and a modulator of endogenous antioxidant enzymes [17].

Nitric oxide, a RNS plays a dual role in physiology and pathology. At physiological concentrations, NO supports vasodilation and immune modulation, but excessive NO can react with superoxide to form peroxynitrite, causing mitochondrial and cellular damage [19-22]. The elevated NO observed post-chloramphenicol exposure is indicative of oxidative-nitrosative stress, which was notably mitigated by antioxidant therapy.

Likewise, GSH serves as a primary intracellular defense molecule, neutralising ROS and acting as a cofactor for various antioxidant enzymes. Depletion of GSH in the chloramphenicol-only group corroborates its role as a sensitive marker of oxidative injury, in agreement with prior findings [23,24]. The significant recovery of GSH in antioxidant-treated groups reinforces the therapeutic promise of Astaxanthin and Quercetin in preserving redox balance.

The present study contributes significant in-vivo evidence to the relatively sparse literature on antioxidant-based interventions for antibiotic-induced hepatotoxicity, especially with chloramphenicol. Although most earlier works have concentrated on metabolic or chemically induced liver damage, The results of the present study highlight that similar oxidative stress pathways are operative in antibiotic-mediated injury, validating antioxidants as potential countermeasures.

Given the affordability and accessibility of both Astaxanthin and Quercetin, their co-administration with hepatotoxic antibiotics like chloramphenicol could offer a practical strategy to mitigate liver damage, particularly in regions lacking access to safer antimicrobial alternatives. These findings support integrating antioxidant adjunct therapy into clinical practice where chloramphenicol remains indispensable, supporting the alternate hypothesis.

Additionally future studies should also focus on exploring the mechanistic pathways involved especially mitochondrial signalling cascades, histopathological and gene expression analysis to supplement biochemical data,dose-response relationships and pharmacokinetics of both antioxidants,evaluating combination therapy in larger cohorts and different models,translational clinical trials to validate safety, optimal dosing, and therapeutic efficacy in humans.

Limitation(s)

While the current study provides valuable insights into the protective effects of Astaxanthin and Quercetin against chloramphenicol-induced hepatotoxicity, certain methodological aspects merit consideration. The use of a relatively small animal cohort may limit the extent to which these findings can be generalised. Additionally, the study was confined to a single animal model and specific dosage levels, which may not fully capture the spectrum of antioxidant efficacy. Although GSH and NO are reliable indicators of oxidative stress, incorporating additional molecular or histological analyses could have enriched the interpretation of liver protection. Furthermore, the 30-day duration restricts conclusions regarding the long-term impact of antioxidant therapy. These aspects offer meaningful directions for future research, including dose optimisation, extended study periods, and broader biomarker analysis.

CONCLUSION(S)

The study demonstrates that both Astaxanthin and Quercetin have significant protective effects against Chloramphenicol-induced oxidative stress in Wistar rats. This is evidenced by the recovery of GSH levels and the reduction of NO levels after antioxidant treatment. Specifically, Quercetin showed a slightly higher efficacy in restoring GSH levels compared to Astaxanthin, although both antioxidants significantly reduced NO levels. To strengthen translational relevance, future studies should explore molecular mechanisms, optimal dosing, long-term outcomes, and confirm efficacy in diverse animal models and clinical settings. If preclinical

results are promising, consider progressing to clinical trials to evaluate the potential therapeutic benefits in humans.

Acknowledgement

We sincerely thank Atmiya University and Zydus research Center for their kind support for the study.

REFERENCES

[1] David S, Hamilton JP. Drug-induced liver injury. US Gastroenterol Hepatol Rev. 2010;6:73-80. PMID: 21874146; PMCID: PMC3160634.
[2] Bjornsson E, Olsson R. Outcome and prognostic markers in severe drug-induced liver disease. Hepatology. 2005;42:481-89.
[3] Reuben A, Koch DG, Lee WM. Drug-induced acute liver failure: Results of a U.S. multicenter, prospective study. Hepatology. 2010;52:2065-76.
[4] Rana P, Aleo MD, Wen X, Kogut S. Hepatotoxicity reports in the FDA adverse event reporting system database: A comparison of drugs that cause injury via mitochondrial or other mechanisms. Acta Pharm Sin B. 2021;11(12):3857-68. Available from: https://doi.org/10.1016/j.apsb.2021.05.028.
[5] National Institute of Diabetes and Digestive and Kidney Diseases. Clinical and research information on drug-induced liver injury. Bethesda (MD): NIDDK; 2012.
[6] Farombi EO, Adaramoye OA, Emerole GO. Influence of chloramphenicol on rat hepatic microsomal components and biomarkers of oxidative stress: Protective role of antioxidants. Pharmacology & Toxicology. 2002;91:129-34. Available from: https://doi.org/10.1034/j.1600-0773.2002.910307.x.
[7] Saba AB, Ola-Davies O, Oyeyemi MO, Ajala O. The toxic effects of prolonged administration of chloramphenicol on the liver and kidney of rats. Afr J Biomed Res. 2000; 3:133-37.
[8] Lautenbach E, Schuster MG, Bilker WB, Brennan PJ. The role of chloramphenicol in the treatment of bloodstream infection due to vancomycin-resistant Enterococcus. Clin Infect Dis. 1998;27:1259-65.
[9] Reiners JJ, Cantu AR, Pavone A, Smith Gardner SC, Laskin DL. Fluorescence assay for per-cell estimation of cytochrome P-450-dependent monooxygenase activities in keratinocyte suspensions and cultures. Anal Biochem. 1990;188:317-24.
[10] Verma BK, Patel HM, Banerjee D. Mitigating chloramphenicol-induced liver toxicity by exploring the therapeutic potential of astaxanthin and quercetin. Sci Rep. 2025;15:33896. Available from: https://doi.org/10.1038/s41598-025-08809-2.
[11] Yamashita E. Let astaxanthin be thy medicine. Pharma Nutrition. 2015;3(4):115-122. Doi: 10.1016/j.phanu.2015.09.001.
[12] Li J, Guo C, Wu J. Astaxanthin in liver health and disease: A potential therapeutic agent. Drug Des Devel Ther. 2020;14:2275-85. Doi: 10.2147/DDDT.S230749. PMID: 32606597; PMCID: PMC7293384.
[13] Wu L, Mo W, Feng J, Li J, Yu Q, Li S, et al. Astaxanthin attenuates hepatic damage and mitochondrial dysfunction in non-alcoholic fatty liver disease by up-regulating the FGF21/PGC-1α pathway. Br J Pharmacol. 2020;177(16):3760-77. Available from: https://doi.org/10.1111/bph.15099.
[14] Sayuti NH, Muhammad Nawawi KN, Goon JA, Mokhtar NM, Makpol S, Tan JK. Preventative and therapeutic effects of astaxanthin on NAFLD. Antioxidants (Basel). 2023;12(8):1552. Available from: https://doi.org/10.3390/antiox12081552.
[15] Liu X, Zhang Y, Liu L, Pan Y, Hu Y, Yang P, et al. Protective and therapeutic effects of nanoliposomal quercetin on acute liver injury in rats. BMC Pharmacol Toxicol. 2020;21:11. Available from: https://doi.org/10.1186/s40360-020-0388-5.
[16] Li L, Lei X, Chen L, Ma Y, Luo J, Liu X, et al. Protective mechanism of quercetin compounds against acrylamide-induced hepatotoxicity. Food Sci Hum Wellness. 2024;13(1):225-40. Available from: https://doi.org/10.26599/FSHW.2022.9250019.
[17] Zhao X, Wang J, Deng Y, Liao L, Zhou M, Peng C, et al. Quercetin as a protective agent for liver diseases: A comprehensive descriptive review of the molecular mechanism. Phytother Res. 2021;35(9):4727-47. Available from: https://doi.org/10.1002/ptr.7104.
[18] Martusevich AK, Davydyuk AV, Martusevich AA, Kovaleva LK. Effects of physiological nitric oxide donor on oxidative metabolism in rat blood. Bull Exp Biol Med. 2017;163:602-04. Available from: https://doi.org/10.1007/s10517-017-3858-z.
[19] Grigor'ev NB, Chechekin GV, Arzamaztsev AP, Levina VI, Granik VG. Nitrogen oxide generation during reduction of nitrofuran antibacterial drugs. Chem Heterocycl Compd. 1999;35(7):788-91. Doi: 10.1007/bf02252100.
[20] Timoshin AA, Gubkina SA, Orlova TR, Ruuge EK, Vanin AF, Chazov EI. Study of the nitric oxide level in the tissues of rat organs and its changes after a long-term inhalation of the air with increased NO content. Dokl Biochem Biophys. 2009;425:110-13. Available from: https://doi.org/10.1134/S1607672909020148.
[21] Miles AM, Wink DA, Cook JC, Grisham MB. Determination of nitric oxide using fluorescence spectroscopy. Methods Enzymol. 1996;268:105-20. Doi: 10.1016/s0076-6879(96)68013-6.
[22] Salvemini D, Ischiropoulos H, Cuzzocrea S. Roles of nitric oxide and superoxide in inflammation. Methods Mol Biol. 2003;225:291-303. Doi: 10.1385/1-59259-374-7:291.
[23] Mytilineou C, Kramer BC, Yabut JA. Glutathione depletion and oxidative stress. Parkinsonism Relat Disord. 2002;8(6):385-87. Doi: 10.1016/s1353-8020(02)00018-4
[24] Merad-Boudia M, Nicole A, Santiard-Baron D, Saillé C, Ceballos-Picot I. Mitochondrial impairment as an early event in the process of apoptosis induced by glutathione depletion in neuronal cells: Relevance to Parkinson's disease. Biochem Pharmacol. 1998;56(5):645-55. Doi: 10.1016/s0006-2952(97)00647-3.
[25] R&D Systems. ELISA HT Glutathione Assay Kit (Catalog Number: 7511-100-K). Minneapolis (MN): R&D Systems.
[26] R&D Systems. Total Nitric Oxide and Nitrate/Nitrite Assay Kit (Catalog Number: KGE001). Minneapolis (MN): R&D Systems.
[27] Guarino MP, Macedo MP. Co-administration of glutathione and nitric oxide enhances insulin sensitivity in Wistar rats. Br J Pharmacol. 2006;147(8):959-65. Doi: 10.1038/sj.bjp.0706691. PMID: 16491098; PMCID: PMC1760719.
[28] Pal G, Mishra HP, Suvvari TK, Tanwar A, Ghosh T, Verma P, et al. Oxidative stress in Wistar rats under acute restraint stress and its modulation by antioxidants and nitric oxide modulators. Cureus. 2023;15(8):e43333. Doi: 10.7759/cureus.43333. PMID: 37701013; PMCID: PMC10493075.
[29] McGill MR, Jaeschke H. Animal models of drug-induced liver injury. Biochimica et Biophysica Acta (BBA) – Molecular Basis of Disease. 2019;1865(5):1031-39. Doi: 10.1016/j.bbadis.2018.08.037.
[30] Nagaraj S, Ramamoorthy K, Sagaya John Paul J, Anand J, Sathuvan M, Arulmurugan P, et al. Antioxidant and anticancer potential of astaxanthin from micro green alga Haematococcus pluvialis during diethyl nitrosamine (DEN)-induced experimental hepatocellular carcinogens in Wistar rats. Clin Case Rep Int. 2022;6:1384.
[31] Edagha IA, Akpan BC, Edem DO, Ataben MA, Bassey BU, Itama RS, et al. Low-dose quercetin at 25 mg/kg ameliorates dolutegravir-lamivudine-tenofovirdisoproxilfumarate-induced cardio-hepato-renal toxicities in Wistar rats. Clin Phytosci. 2024;10:10. Available from: https://doi.org/10.1186/s40816-024-00377-8.
[32] Islam MA, Al Mamun MA, Faruk M, Ul Islam MT, Rahman MM, Alam MN, et al. Astaxanthin ameliorates hepatic damage and oxidative stress in carbon tetrachloride-administered rats. Pharmacognosy Res. 2017;9(Suppl 1):S84-91. Doi: 10.4103/pr.pr_26_17. PMID: 29333048; PMCID: PMC5757332.
[33] Szczepanik K, Oczkowicz M, Dobrowolski P, Świątkiewicz M. The protective effects of astaxanthin (AST) in the liver of weaned piglets. Animals. 2023;13(20):3268. Doi: 10.3390/ani13203268.
[34] Katsaros I, Sotiropoulou M, Vailas M, Papachristou F, Papakyriakopoulou P, Grigoriou M, et al. The effect of quercetin on non-alcoholic fatty liver disease (NAFLD) and the role of Beclin1, P62, and LC3: An experimental study. Nutrients. 2024;16(24):4282. Doi: 10.3390/nu16244282.
[35] Abushady EA, Elagaty SM, Nassef NA, Abdelhamid GS. The potential hepatoprotective effect of quercetin on cholestatic liver injury in rats. QJM. 2020;113(Suppl 1): Hcaa065.002. Doi: 10.1093/qjmed/hcaa065.002.

PARTICULARS OF CONTRIBUTORS:
1. Research Scholar, Department of Biotechnology, Atmiya University, Rajkot, Gujarat, India.
2. Research Scientist, Zydus Research Center, Gujarat, India.
3. Assistant Professor, Department of Biotechnology, Atmiya University, Rajkot, Gujarat, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Debashis Banerjee,
Assistant Professor, Department of Biotechnology, Atmiya University,
Rajkot-360005, Gujarat, India.
E-mail: debashis.banerjee@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Dec 24, 2024
- Manual Googling: Jul 21, 2025
- iThenticate Software: Jul 24, 2025 (8%)

ETYMOLOGY: Author Origin

EMENDATIONS: 7

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? NA
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Dec 23, 2024**
Date of Peer Review: **May 09, 2025**
Date of Acceptance: **Jul 26, 2025**
Date of Publishing: **Feb 01, 2026**